Crystal structure of NGF in complex with the ligand-binding domain of the TrkA receptor

Christian Wiesmann, Mark H. Ultsch, Abraham M. de Vos.
Department of Protein Engineering, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080

INTRODUCTION

Nerve growth factor (NGF) is the best characterized member of the neurotrophin family, which is involved in a variety of signaling events such as cell differentiation and survival, growth cessation, and apoptosis of neurons [1]. These biological functions are mediated through binding to two classes of cell-surface receptors, the Trks and p75 [2]. TrkA is a receptor tyrosine kinase and binds to NGF with high affinity [3]. Of the five domains comprising its extracellular portion, the immunoglobulin-like domain proximal to the membrane (TrkA-d5) is necessary and sufficient for NGF binding [4]. We have determined the crystal structure of human NGF in complex with human TrkA-d5 at 2.2 Å resolution, using data collected at the Berkeley Advanced Light Source.

RESULTS AND DISCUSSION

The asymmetric unit of the crystals contains one complex consisting of two NGF monomers that assemble in a parallel arrangement to form the active dimer and two copies of TrkA-d5. Overall, the NGF dimer is similar to the structure of mouse NGF solved by McDonald *et al.* [5], but the N-terminal segment undergoes a major change upon receptor binding (below). TrkA-d5 folds into an immunoglobulin-like domain of the I-set family [6], consisting of a β-sandwich with two four-stranded β-sheets (ABED and CC'FG). It is noteworthy that this domain has several unexpected structural features, such as an unusual conformation of the AB loop and a disulfide bridge exposed at the surface of the ABED sheet.

Each interface between NGF and TrkA-d5 buries a total of about 2200 $Å^2$ of solvent-accessible surface. The interface can be divided into two patches of similar size. One patch involves the central β-sheet that forms the core of the homodimeric NGF molecule and the loops on the C-terminal pole of TrkA-d5, including the unusual AB-loop. The residues comprising this patch are largely conserved, both among the neurotrophin ligands and among their receptors. We believe this patch is utilized for complex formation by all members of the family. The second patch consists of the N-terminal residues of NGF, which were disordered in the previously reported structures of NGF [5] but adopt a helical conformation upon complex formation, packing against the disulfide bond at the surface of the ABED sheet of TrkA-d5. The residues in this patch are poorly conserved among ligands as well as receptors; furthermore, swaps of the N-

terminus of the ligands have resulted in bi-specific neurotrophins able to bind to and signal through several receptors [7]. Therefore, this second patch controls the specificity among the family members. The structure is consistent with the mutagenesis data not only for NGF but also for the other ligands in the neurotrophin family [8,9]. Thus, in contradiction to current belief that these ligands interact with their receptors in different binding modes [10], our structure strongly suggests that the overall manner of binding is conserved throughout the family.

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Principal investigator: Abraham M. de Vos, Department of Protein Engineering, Genentech, Inc. Email: devos@gene.com. Telephone: 650-225-2523.